



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification <sup>7</sup> : C12N 15/67, 15/80, 15/81, C12P 21/02, C12N 15/55, 9/18, 9/36, 15/13, C07K 16/00</p>	<p>A3</p>	<p>(11) International Publication Number: <b>WO 00/05389</b>  (43) International Publication Date: 3 February 2000 (03.02.00)</p>
<p>(21) International Application Number: PCT/EP99/04919  (22) International Filing Date: 8 July 1999 (08.07.99)  (30) Priority Data: 98202432.5 20 July 1998 (20.07.98) EP  (71) Applicant (for all designated States except AU BB CA CY GB GD GH IE IL KE LK LS MN MW NZ SD SG SZ TT UG ZA): UNILEVER N.V. [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL).  (71) Applicant (for AU BB CA CY GB GD GH IE IL KE LK LS MN MW NZ SD SG SZ TT UG ZA only): UNILEVER PLC [GB/GB]; Unilever House, Blackfriars, London EC4P 4BQ (GB).  (72) Inventors: FRENKEN, Leon, Gerardus, Joseph; Unilever Re- search Colworth, Colworth House, Sharnbrook, Bedford, Bedfordshire MK44 1LQ (GB). SAGT, Cees; Univer- siteit van Utrecht, Heidelberglaan 8, NL-3584 CS Utrecht (NL). VERKLEIJ, Arie, J.; Universiteit van Utrecht, Hei- delberglaan 8, NL-3584 CS Utrecht (NL). VERRIPS, Cor- nelis, Theodorus; Unilever Research Vlaardingen, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL).</p>		<p>(74) Agent: WURFBAIN, Gilles, L.; Unilever N.V., Patent Depart- ment, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL).  (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims</i> <i>and to be republished in the event of the receipt of amendments.</i>  (88) Date of publication of the international search report: 2 June 2000 (02.06.00)</p>
<p>(54) Title: PRODUCTION OF PROTEINS  (57) Abstract  A method for producing a heterologous protein in a lower eukaryotic cell, followed by secretion of said protein from said cell, said protein comprising at least one hydrophobic stretch, wherein the protein is glycosylated such as to increase the level of secretion of the protein, without substantially affecting the functionality of the protein and wherein at least one N-glycosylation group is located between the N-terminus of the protein and the first hydrophobic stretch.</p>		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 99/04919

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/67 C12N15/80 C12N15/81 C12P21/02 C12N15/55  
C12N9/18 C12N9/36 C12N15/13 C07K16/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 C12N C12P C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 05228 A (CREATIVE BIOMOLECULES INC.; GOVERNMENT (US); JOST; SEGAL; HUSTON) 22 February 1996 (1996-02-22) cited in the application	1-7, 9-14
Y	abstract page 3, line 31 -page 7, line 22 page 10, line 31 -page 17, line 32 page 18, line 4-7	5
Y	WO 94 14963 A (UNILEVER PLC (GB); UNILEVER N.V. (NL); EGMOND; VAN DER HIJDEN ET AL.) 7 July 1994 (1994-07-07) cited in the application page 18, line 14-31	5

-/-

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "a" document member of the same patent family

Date of the actual completion of the international search

28 March 2000

Date of mailing of the international search report

04/04/2000

Name and mailing address of the ISA  
European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Macchia, G

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SAGT C.M.J. ET AL.: "Impaired secretion of a hydrophobic cutinase by <i>Saccharomyces cerevisiae</i> correlates with an increased association with immunoglobulin heavy-chain binding protein (BiP)" APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 64, no. 1, January 1998 (1998-01), pages 316-324, XP002088542 cited in the application abstract	5
X	EP 0 324 274 A (CHIRON CORPORATION (US); TEKAMP-OLSON P.) 19 July 1989 (1989-07-19) page 2, line 40 -page 3, line 24 page 6, line 54 -page 7, line 38	1-7,9-14
X	EP 0 394 951 A (TAKEDA CHEMICAL INDUSTRIES LTD. (JP); SENOO M.; SASADA R.; IGARASHI K.) 31 October 1990 (1990-10-31) cited in the application abstract page 2, line 23 -page 3, line 4 page 12, line 21 -page 16, line 29	1-4,6,7,9-14
A	ZHU H. ET AL.: "Glu-96 of basic fibroblast growth factor is essential for high affinity receptor binding" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 37, 15 September 1995 (1995-09-15), pages 21869-21874, XP002073993 page 21871, right-hand column; figure 2	1,4
X	EP 0 704 527 A (PLIVA (YU); MESTRIC S; PUNT P.J; VALINGER R; VAN DEN HONDEL C.A.M.J.J) 3 April 1996 (1996-04-03) cited in the application page 3, line 30-32	1-4,6,8-14
A	BLOND-ELGUINDI S. ET AL.: "Affinity panning of a library of peptides displayed on bacteriophages reveals the binding specificity of BiP" CELL, vol. 75, 1993, pages 717-728, XP002087932 cited in the application abstract page 718; figure 1	2
	-/-	

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/04919

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BEKKERS A.C.A.P.A. ET AL.: "The use of genetic engineering to obtain efficient production of porcine pancreatic phospholipase A2 by <i>Saccharomyces cerevisiae</i> " BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1089, 1991, pages 345-351, XP002088381 abstract	1,7
A	CAPLAN S. ET AL.: "Glycosylation and structure of the yeast MF-alpha-1 alpha-factor precursor is important for efficient transport through the secretory pathway" JOURNAL OF BACTERIOLOGY, vol. 173, no. 2, January 1991 (1991-01), pages 627-635, XP002088382 abstract	1,7
A	PRAETORIUS-IBBA M. ET AL.: "Homologous recombination partly restores the secretion defect of underglycosylated acid phosphatase in yeast" CURRENT GENETICS, vol. 32, no. 3, 1997, pages 190-196, XP002088383 abstract	1,7

## INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. Application No.

PCT/EP 99/04919

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9605228 A	22-02-1996	US 5888773 A	30-03-1999
		AU 700475 B	07-01-1999
		AU 3244695 A	07-03-1996
		CA 2197232 A	22-02-1996
		EP 0779897 A	25-06-1997
		JP 10504458 T	06-05-1998
WO 9414963 A	07-07-1994	AU 5699994 A	19-07-1994
		BR 9307678 A	31-08-1999
		CA 2150837 A	07-07-1994
		CN 1090328 A	03-08-1994
		CZ 9501578 A	13-12-1995
		EP 0679188 A	02-11-1995
		HU 71325 A	28-11-1995
		JP 8504588 T	21-05-1996
		PL 309388 A	02-10-1995
		SK 79595 A	08-11-1995
		ZA 9309415 A	15-06-1995
EP 0324274 A	19-07-1989	AT 97441 T	15-12-1993
		AU 2765088 A	06-07-1989
		DE 3885728 D	23-12-1993
		DE 3885728 T	10-03-1994
		DK 728588 A	01-07-1989
		ES 2059547 T	16-11-1994
		FI 886008 A	01-07-1989
		HK 139994 A	16-12-1994
		IE 62087 B	14-12-1994
		JP 2002339 A	08-01-1990
		JP 2793215 B	03-09-1998
		NO 178829 B	04-03-1996
		PT 89367 A,B	29-12-1989
		US 5602034 A	11-02-1997
EP 0394951 A	31-10-1990	CA 2015313 A	26-10-1990
		JP 3061494 A	18-03-1991
		US 5464943 A	07-11-1995
EP 0704527 A	03-04-1996	HR 940432 A	31-08-1997
		BG 62336 B	31-08-1999
		BG 99844 A	29-02-1996
		CA 2155451 A	06-02-1996
		CN 1126761 A	17-07-1996
		CZ 9501999 A	14-02-1996
		PL 309882 A	19-02-1996
		SI 9500250 A	29-02-1996
		SK 97195 A	07-02-1996